

Preparation and characterization of magnetic poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone) microspheres

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Abstract In this article, nano-magnetite particles (ferrofluid, Fe_3O_4) were prepared by chemical co-deposition method. A series of biodegradable triblock poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone) (PCL-PEG-PCL, PCEC) copolymers were synthesized by ring-opening polymerization method from ϵ -caprolactone (ϵ -CL) initiated by poly(ethylene glycol) diol (PEG) using stannous octoate as catalyst. And the magnetic PCEC composite microspheres were prepared by solvent diffusion method. The properties of the ferrofluid, PCEC copolymer, and magnetic PCEC microspheres were studied in detail by SEM, VSM, XRD, Malvern Laser Particle Sizer, $^1\text{H-NMR}$, GPC, and TG/DTG. Effects of macromolecular weight and concentration of polymer, and the time for ultrasound dispersion on properties of magnetic microspheres were also investigated. The obtained magnetic PCEC microspheres might have great potential application in targeted drug delivery system or cell separation.

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Introduction

Magnetic polymeric microspheres, as a type of functional materials, have been applied in drug targeting and controlled release system [1, 2], cell separation [3], immunoassay [4], protein or enzyme immobilization [5], nucleic acid concentration [6], and etc. Several methods, such as emulsion polymerization [7], dispersion polymerization [8], suspension polymerization [9], micro-emulsion polymerization [10], solvent diffusion [11], emulsion diffusion [12], self-emulsion solvent diffusion [13], and solvent evaporation methods [14], had been reported to prepare magnetic polymeric microspheres. Many polymers, including polylactide [15], PLA-PEG copolymer [16], styrene/divinylbenzene copolymers [17], and etc, had been used in this field. Biodegradable poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone) (PCL-PEG-PCL, PCEC) copolymer was widely used as controlled drug delivery system [18], but its application in magnetic polymer composite microspheres was seldom found in the literature.

In this study, we synthesized a series of PCEC copolymers, and prepared the magnetic PCEC composite microspheres by solvent diffusion method. The obtained magnetite PCEC composite microspheres might have great potential application in targeted drug delivery system.

Experimental

Materials

Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), sodium hydroxide (NaOH), antioxidant 1010 and sodium dodecyl sulphate (SDS) were purchased from TianJing Fine Chemicals, China.

Poly(ethylene glycol)s (PEG, $M_n = 4,000$ and $8,000$), which were purchased from KeLong Chemicals, Chengdu, China, were used after vacuum dried at $90\text{ }^\circ\text{C}$ for 3 h. ϵ -Caprolactone (ϵ -CL) and stannous octoate ($\text{Sn}(\text{Oct})_2$) were purchased from Sigma (USA).

All the chemicals used in this work were all analytical pure grade, and used as received except PEG.

Preparation of ferrofluid

6.25 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 3.98 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were put in 120 mL of 5 wt% SDS aqueous solution, and the obtained solution was named Solution A. 5 g of NaOH was dissolved in 120 mL of 5 wt% SDS aqueous solution, and the resultant solution was called Solution B. Solution B was then transferred into a glass ampoule kept at $70\text{ }^\circ\text{C}$, and stirred at a speed of about 300 rpm. Then, Solution A was dropped into Solution B under nitrogen atmosphere. The mixed solution was stirred for 1 h. During this period, color of the solution changed from transparent to black. Then, the magnetic particles were collected by magnetic-separation. The resultant magnetic particles were rinsed with distilled water for three times to wash off free SDS, NaCl and NaOH. At last, the suspension containing magnetite particles was adjusted to 6% wt and ultrasound-dispersed for further use.

Preparation of PCL-PEG-PCL (PCEC) copolymers

PCEC copolymers were prepared by introducing calculated amount of ϵ -CL and PEG under nitrogen atmosphere into a dry glass ampoule, and several drops of $\text{Sn}(\text{Oct})_2$ was added. The ampoule was kept at $130\text{ }^\circ\text{C}$. During polymerization, the system was stirred slowly, and the viscosity increased with time. 6 h later, the system was rapidly elevated to $180\text{ }^\circ\text{C}$ under vacuum for another 20 min. After cooled to room temperature under nitrogen atmosphere, PCEC copolymer was first dissolved in methylene chloride and reprecipitated from the filtrate using excess cold petroleum ether. Then the mixture was filtered and vacuum-dried to constant weight. The purified PCEC copolymers were kept in air-tight bags in desiccator before use.

Preparation of porous magnetic PCEC microspheres

0.5 g of PCEC was dissolved in 15 mL of dimethyl sulfoxide (DMSO), and 0.4 mL of ferrofluid (6 wt%) was added. Then, the suspension was dispersed by ultrasound (JY92-2D, Ningbo Scientz Biotechnology Co., China) at 200 W. 5 min later, 1.5 g of PEG8000 was added into this solution, and it was ultrasound-dispersed for another 3 min. And then, the well-dispersed suspension was dropped into 100 mL of 1 wt% PVA aqueous solution under ultrasonic

dispersion (Benchtop 20L, Medisafe UK Ltd, UK). After 20 min, the porous magnetic PCEC composite microspheres were prepared successfully.

The obtained magnetic PCEC composite microspheres were collected by centrifugation (Optima L-80XP, Beckman Coulter, Germany) at a speed of 10,000 rpm, and then magnetic-separated. The composite microspheres were rinsed with distilled water and centrifuged three times to wash off remained PVA, and magnetic-separated for two times to remove the microspheres without magnetism. Then, the microspheres were lyophilized and stored at $4\text{ }^\circ\text{C}$.

^1H nuclear magnetic resonance ($^1\text{H-NMR}$)

$^1\text{H-NMR}$ spectra (in CDCl_3) were recorded on Varian 400 spectrometer (Varian, USA) at 400 MHz using tetramethylsilane as an internal reference standard.

Gel permeation chromatography (GPC)

In this paper, GPC (Agilent 110 HPLC, America) was used to determine macromolecular weight and distribution of PCEC copolymers. Polymer samples were dissolved in freshly distilled tetrahydrofuran (THF) at a concentration of 1–2 mg/mL. The THF was eluted at a rate of 1.0 mL/min through two Waters Styragel HT columns and a linear column. The internal and column temperatures were kept at $35\text{ }^\circ\text{C}$. The macromolecular weight was calculated from elution volume of polystyrenes with narrow molecular weight distribution.

Scanning electron microscopy (SEM)

SEM (JSM-5900LV, JEOL, Japan) was employed to investigate surface morphology of magnetite particles and magnetic PCEC composite microspheres. The samples were sputtered with gold before observation.

Laser diffraction particle size analyzer

The particle sizes and distributions were determined by Malvern Nano-ZS 90 laser particle size analyzer. The measurement temperature was kept at $25\text{ }^\circ\text{C}$.

X-ray diffraction (XRD)

A crystallographic study was performed on magnetite particles by X-ray Diffractometer (DX-2000, DanDong Fangyuan Instrument Company, China) using $\text{CuK}\alpha$ radiation. The sample's d_{space} was calculated according to Bragg's law, and which was compared with ASTM X-ray Diffraction data.

Thermogravimetric analysis (TG/DTG)

Thermogravimetric measurements were characterized on thermogravimetric analyzer (WRT-2P, ShangHai Balance Instrument Factory, China) under nitrogen atmosphere at a heating rate of 10 °C/min from room temperature to 700 °C.

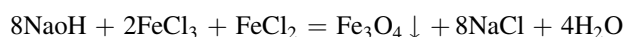
VSM magnetometer

VSM magnetometer (JDM-13, JiLin University, China) was used to measure magnetic properties of the ferrofluid and magnetic PCEC composite particles.

Result and discussion

Preparation and characterization of ferrofluid

Magnetite (Fe₃O₄) nanoparticles were formed by chemical co-deposition method and the chemical reaction equation for preparation of Fe₃O₄ was shown as follows:



As we know, many different types of iron oxides, such as magnetite (Fe₃O₄), maghemite (γ -Fe₂O₃), hematite (α -Fe₂O₃), and martite (Fe₂O₃), exhibit magnetic properties [19]. The crystallographic structure of iron oxide type was determined by XRD. Figure 1 showed XRD pattern of magnetite particles. The experimental d_{space} results were listed in Table 1, which were consistent with ASTM data of Fe₃O₄. So, according to d_{space} results and preparation method, the conclusion could be made that the obtained iron oxide particles were magnetite (Fe₃O₄).

Figure 2 presented M–H curve of the obtained ferrofluid, and its saturated magnetization was 1.5639 emu/43mg = 36.37 emu/g. The magnetization decreased from plateau value to zero when magnetic field intensity decreased, which implied that the prepared ferrofluid in this paper had no remanence. In another words, the obtained magnetite particle was paramagnetic.

The particle size distribution of the magnetic Fe₃O₄ microparticles was shown in Fig. 3. According to Fig. 3, the average particles size of magnetite particles was ca.79 nm, and the particle size distribution was very narrow. Scanning electron micrograph of magnetite particles was presented in Fig. 4, according to which, we could find that the average size of Fe₃O₄ particles was about 50 nm and they were spherical. Figures 3 and 4 showed that we could prepare magnetite nanoparticles by chemical co-deposition method.

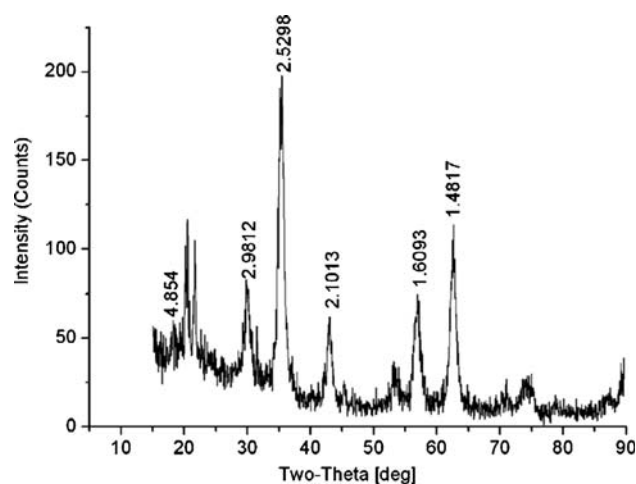


Fig. 1 XRD spectrum of the magnetite particles

Table 1 Experimental $d(\text{\AA})$ spacings from XRD patterns, $d(\text{exp})$ and d spacings from ASTM cards for iron oxide (Fe₃O₄) synthesized

2θ (exp)	d_{space} (Experimental data)	d_{space} (Standard data)
18.300	4.854	4.852
30.120	2.9812	2.967
35.480	2.5298	2.532
43.120	2.1013	2.0993
57.020	1.6093	1.6158
62.620	1.4817	1.4845

Characterization of PCEC copolymer

In this paper, PCEC triblock copolymers were prepared by ring-opening polymerization of ϵ -CL initiated by PEG-diol (HO-PEG-OH) according to Scheme 1. Macromolecular weight of PCEC copolymer was controlled by ϵ -CL/PEG ratio. As shown in Table 2, with increase in ϵ -CL/PEG, macromolecular weight of PCEC copolymers increased

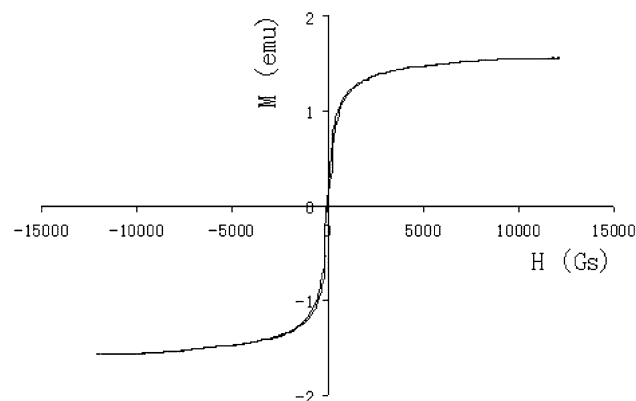


Fig. 2 M–H curve of the prepared magnetite nano-particles (sample weight was 43 mg)

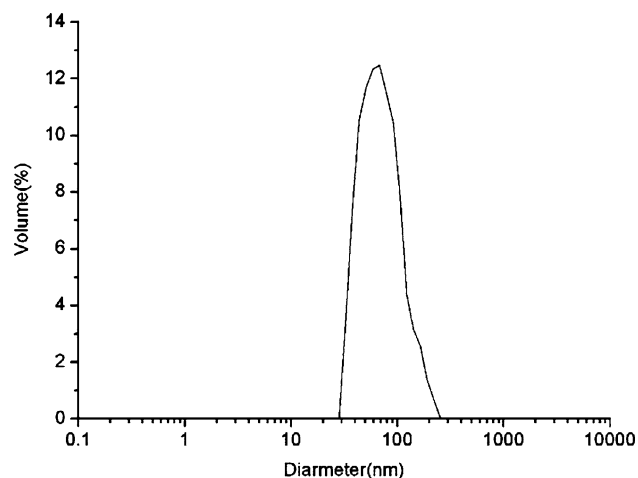


Fig. 3 Particle size distribution of prepared magnetite nano-particles

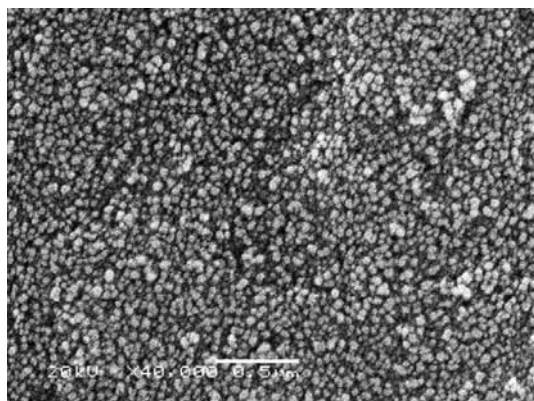
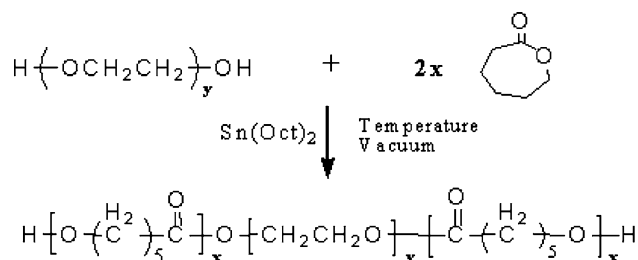


Fig. 4 SEM photomicrograph of the prepared magnetite nano-particles

then. The prepared PCEC copolymers in this work were listed in Table 2.

The prepared PCEC copolymers were characterized by $^1\text{H-NMR}$. Figure 5 was the typical $^1\text{H-NMR}$ spectrum of PCEC-5 copolymers. In this figure, peaks “f”, “e”, and “b” were assigned to methylene protons of $-(\text{CH}_2)_3-$, $-\text{OCCH}_2-$ and $-\text{CH}_2\text{OOC}-$ in PCL units, respectively. The peak “d” was attributed to methylene protons of PEG



Scheme 1 Synthesis scheme of PCEC copolymers

oxyethylene units. The weak peaks “a” and “c” were attributed to methylene proton of PEG end units. From Fig. 5, we could find that PCEC was prepared successfully.

Figure 6 is the typical GPC curve of PCEC-4 copolymer. According to Fig. 6, we could find that only a single peak existed, which indicated the mono-distribution of macromolecular weight and absence of any homopolymer of ϵ -CL or PEG, and also implied that no transesterification or backbiting reactions occurred during polymerization [18].

Preparation of porous magnetic PCEC microspheres

Because DMSO was miscible with water even at high content, and it could dissolve PCEC, but PCEC could not be dissolved in water, solvent diffusion method was employed to prepare PCEC composite microspheres in this paper. When PCEC/DMSO solution was dropt into aqueous PVA solution under ultrasound dispersion, the PCEC/DMSO micro-drops formed and then the PCEC microspheres were obtained due to the extraction of DMSO.

In this work, five kinds of PCEC copolymers were used to prepare magnetic polymeric composite microspheres. SEM was employed to observe microspheres surface morphology. Figures 7 and 8 were SEM micrographs of magnetic PCEC microspheres.

Figure 7a presented SEM micrograph of PCEC-1/ Fe_3O_4 composite microparticles, which indicated that the microparticles were very small. The average particle size of the particles was about 239 nm determined by MALVERN laser particle sizer. But according to SEM micrograph, average particle size was smaller than 100 nm, which was much smaller than the result determined by Malvern laser particle sizer. The morphology of PCEC-2/ Fe_3O_4 composite particles was shown in Fig. 7b. There were some particles at size of 1–2 μm and many irregular substances mingled with them. The average size of PCEC-2/ Fe_3O_4 particles was about 610 nm. Figure 7c, d were SEM micrographs of PCEC-3/ Fe_3O_4 and PCEC-4/ Fe_3O_4 microparticles respectively. The shape of PCEC-4/ Fe_3O_4 microspheres was nearly spherical, but PCEC-3/ Fe_3O_4 composite particles were less spherical. When PCEC-5/ Fe_3O_4 composite particles were prepared, some particles at abnormal big size could be observed with naked eyes directly. And such abnormal big microspheres had little magnetic responsivity. So, through magnetic separation, two types of particles at different particle sizes were obtained. According to Fig. 7e, PCEC-5/ Fe_3O_4 composite particles were about several microns. These abnormal big microspheres without magnetic properties were shown in Fig. 7f. These microspheres were in strange shape even alike apple. The irregular shape observed when a high molecular weight PCEC polymer is used, which might be

Table 2 Polymerization of ε-CL with different ratio of PEG (M_w = 4,000), and characterization of triblock copolymers

Sample	M _{n, PEG}	ε-CL:PEG (In feed, W/W%)	M _n ^a (Theoretical)	M _{n, GPC} ^b	M _w ^c /M _n ^b
PCEC-1	4,000	2:1	12,000	7,395	1.5490
PCEC-2	4,000	4:1	20,000	10,642	1.4072
PCEC-3	4,000	9:1	40,000	18,853	1.6236
PCEC-4	4,000	19:1	80,000	26,382	1.7093
PCEC-5	4,000	24:1	100,000	35,895	1.7839

^a Calculated according to feed ratio

^b Number-average molecular weight measured by GPC

^c Weight-average molecular weight measured by GPC

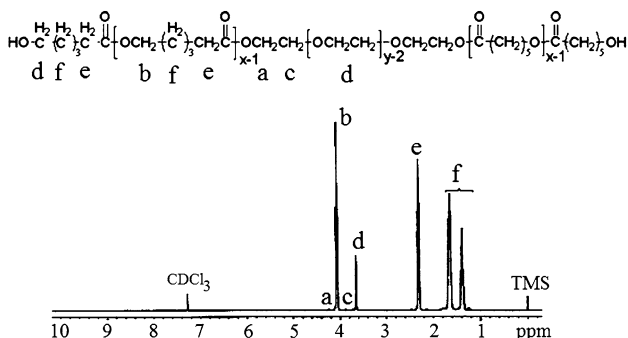
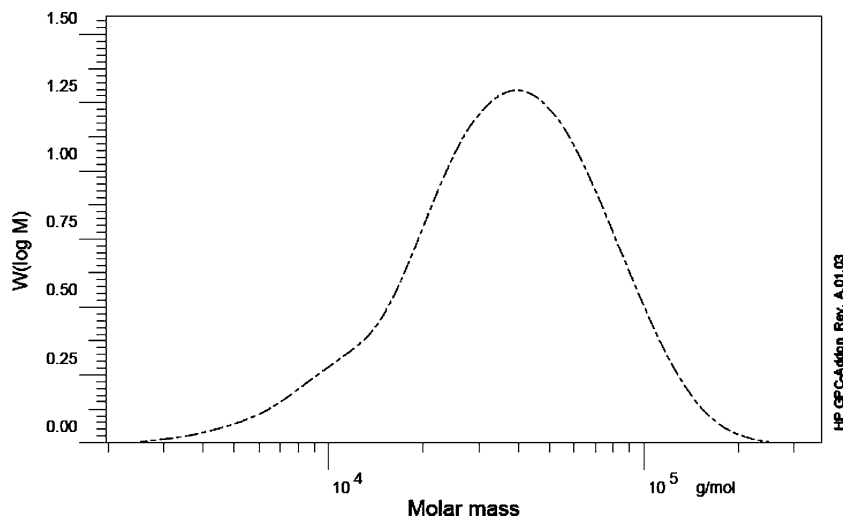


Fig. 5 ¹H-NMR spectra of the PCEC-5 copolymer

due to formation of a ‘skin’ around the forming particle. This skin effect prevents the gradual compaction of the microparticles leading to a bigger particle size and a more porous structure. Furthermore, since the formation of the skin the particle may undergo a mechanical collapse which leading to irregular shape. Probably, when using high molecular weigh PCEC, the polymer ‘skin’ around the formed particle is more compact. In order to overcome this problem, a slower solvent extraction should be performed.

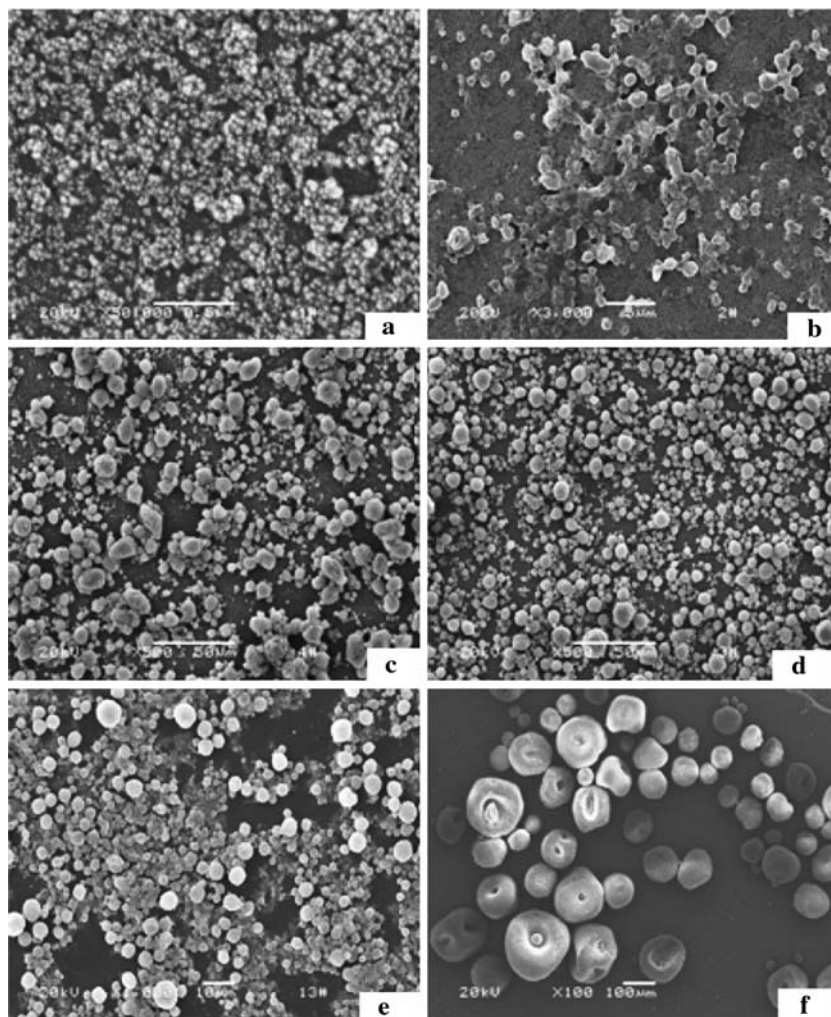
Fig. 6 GPC curve of PCEC-4 copolymer



From Fig. 7, we could find that particle size distributions of the prepared PCEC-2/Fe₃O₄, PCEC-3/Fe₃O₄, PCEC-4/Fe₃O₄ and PCEC-5/Fe₃O₄ composite particles were not narrow. With increase in macromolecular weight of PCEC, the average particle size increased then according to Table 3. It might be due to the existence of surface interaction between polymer solution and water. As shown in Table 2, macromolecular weight of PCEC increased with increase in ε-CL/PEG, and which resulted in increase in hydrophobicity of PCEC copolymer. As a result, the interfacial tension between polymer solution and water increased, and then the average particle size of obtained particles became larger. With the increase in macromolecular weight of PCEC copolymer, the shape of resultant magnetic PCEC composite microspheres became regular, which could be seen from Fig. 8a, c and e.

From Fig. 8b, d and f, pores on surface of the microspheres were observed and sizes of the pores were smaller than 50nm. But in Fig. 8g and h, pores on the microspheres surface and inside were observed. So, it might be concluded that porous microspheres could be prepared by solvent diffusion method with the addition of PEG.

Fig. 7 SEM graphs of magnetic copolymer composite microspheres. (a) $\text{Fe}_3\text{O}_4/\text{PCEC-1}$ (b) $\text{Fe}_3\text{O}_4/\text{PCEC-2}$ (c) $\text{Fe}_3\text{O}_4/\text{PCEC-3}$ (d) $\text{Fe}_3\text{O}_4/\text{PCEC-4}$ (e) and (f) $\text{Fe}_3\text{O}_4/\text{PCEC-5}$



TG/DTG was used to determine content of magnetite particle in such polymeric composite microparticles. When the temperature reached ca.500 °C, the polymers had been burnt off, but the magnetite particles did not change. So the content of magnetite particles could be determined according to Eq 1:

$$H(\%) = (W_2/W_1) \times 100. \quad (1)$$

where H is content of Fe_3O_4 in magnetic PCEC microspheres; W_1 is the total weight of sample; W_2 is the residual weight of sample at 500 °C.

Figure 9 was TG/DTG spectrum of magnetite/PCEC-4 composite microspheres. As shown in Fig. 9, there was a visible exothermic peak at about 420 °C and a small flat roof at about 475 °C, appearing in DTG curve. According to Fig. 9, content of magnetite particles in magnetite/PCEC-4 composite was about 4.63% wt. Effect of macromolecular weight of PCEC on content of magnetite particles in $\text{Fe}_3\text{O}_4/\text{PCEC}$ microspheres was shown in Table 3 and Fig. 10. It indicated that $\text{Fe}_3\text{O}_4/\text{PCEC-1}$ composite

microspheres with magnetite content of 83.78% might be core-shell structure (magnetite particles as core, and PCEC as shell). With increase in macromolecular weight of PCEC, the content of magnetite decreased then. But for $\text{Fe}_3\text{O}_4/\text{PCEC-5}$ microsphere, due to its high molecular weight, lots of microspheres without magnetite particles were obtained. After magnetic separation, these particles without magnetic particles were discarded. As a result, the amount of magnetic particles was small, but the magnetite content of such magnetic particles was higher than that in feed. That's to say, the experimental value of Fe_3O_4 was higher than the theoretical value.

The magnetic properties of the prepared porous magnetic PCEC composite microsphere were also detected by VSM. The M-H curve of $\text{Fe}_3\text{O}_4/\text{PCEC-4}$ composite microsphere (S-4 sample) was shown in Fig. 11. And it indicated that the polymeric composite microspheres had great magnetic responsivity. And the composite microspheres with magnetic content of 4.63 wt% also had paramagnetism.

Fig. 8 SEM graphs of PCEC-3/ Fe_3O_4 (a, b) PCEC-4/ Fe_3O_4 (c, d) and PCEC-5/ Fe_3O_4 (e–h) composite microspheres at high magnification

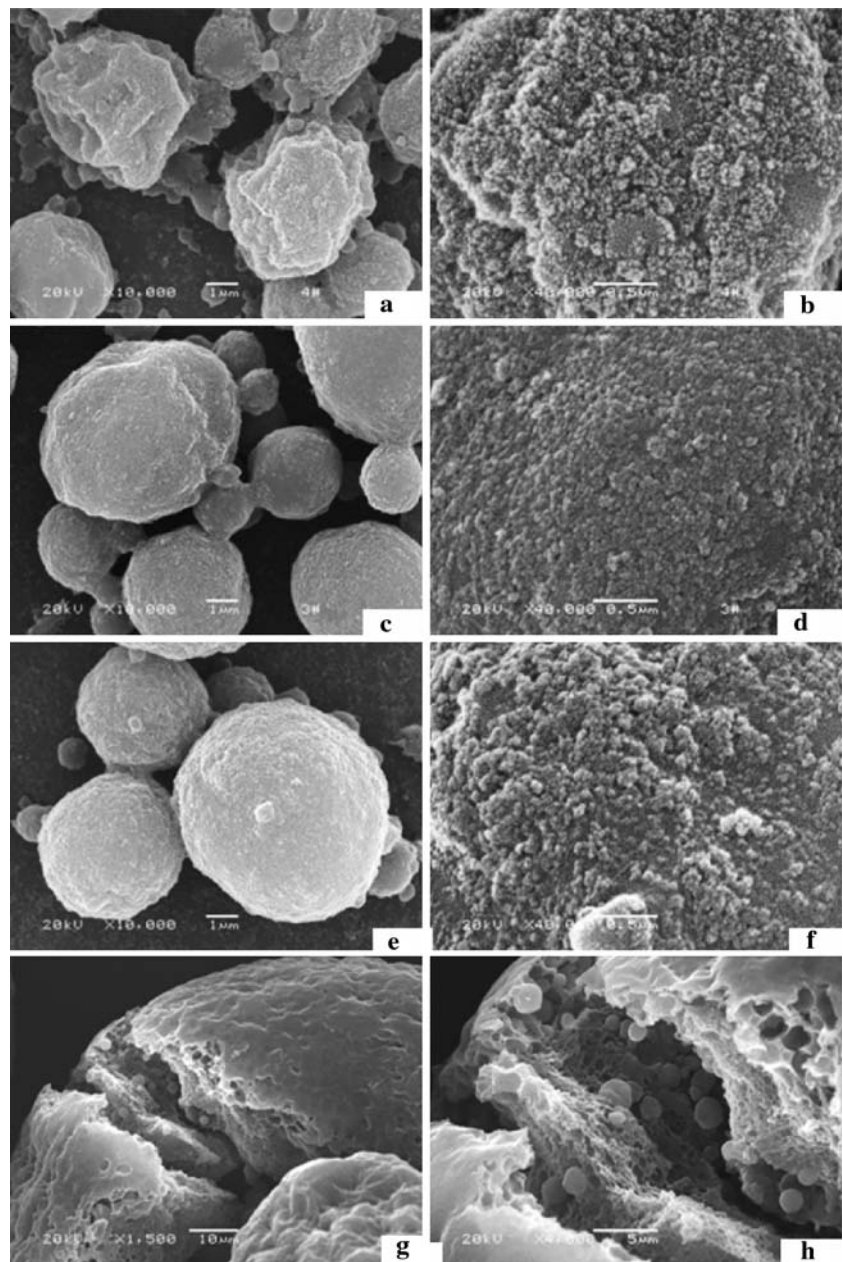


Table 3 Effect of macromolecular weight on magnetic PCEC composite particles

Sample	Polymer	Time of ultrasound dispersion (min)	Theoretical Fe_3O_4 content (wt%) ^a	Experimental Fe_3O_4 content (wt%) ^b	Diameter (nm)
S-1	PCEC1	5	4.8	83.78	239
S-2	PCEC2	5	4.8	44.64	610
S-3	PCEC3	5	4.8	5.49	3,125
S-4	PCEC4	5	4.8	4.63	3,640
S-5	PCEC5	5	4.8	18.51	4,080

^a The magnetite particles content in feed

^b Determined by TG–DTG

At the same time, PCEC-4 was also employed to investigate the effects of ultrasound time and the polymer concentration on the size of obtained microsphere. When the ultrasound time was kept at 10 min, 30 min or

60 min, the size of the obtained microsphere did not change significantly. It indicated that the solvent extraction speed was fast and the microsphere could form in 10 min.

Fig. 9 TG–DTG spectra of the magnetite/PCEC-4 composite microspheres

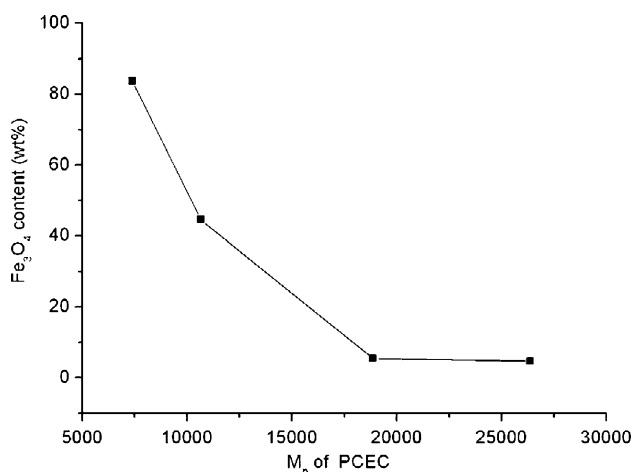
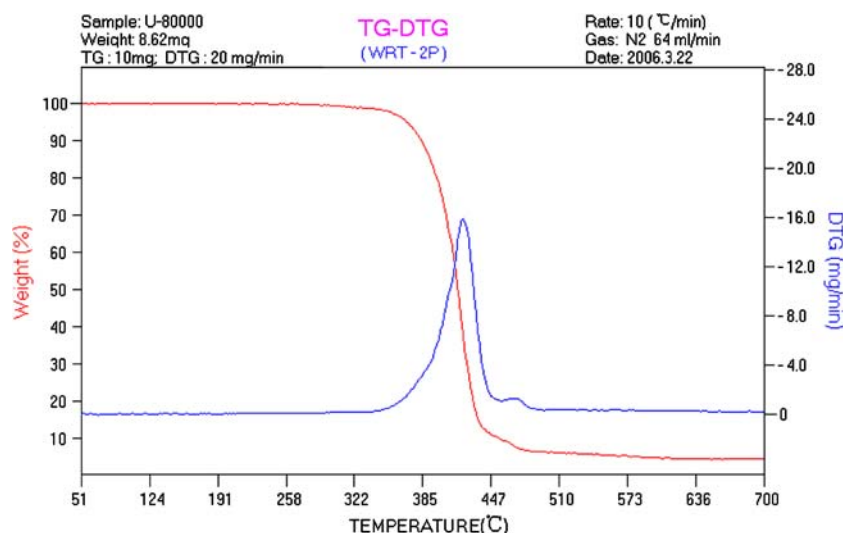


Fig. 10 Effect of macromolecular weight of PCEC on the content of magnetic particles in microspheres

Meanwhile, when polymer concentration was kept at 1% and 2% (w/v), the average particle size of the microspheres were 2,356 nm and 3,011 nm respectively. So, the particle

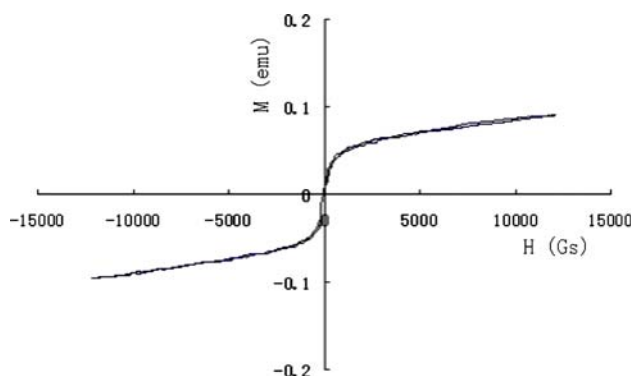


Fig. 11 M–H curve of PCEC-4/magnetite composite microsphere (S-4, sample weight was 30 mg)

size of prepared microsphere increased with the increase of polymer concentration.

Conclusion

In this paper, magnetite nanoparticles and PCEC copolymers were successfully synthesized. And then magnetic PCEC microsphere incorporated nano-ferrite was prepared by solvent extraction method. The effect of molecular weight and concentration of PCEC copolymers, and the time for ultrasound dispersion on the properties of obtained microspheres were assessed. The demonstrated magnetic PCEC microspheres might have great potential application in biomedical field such as drug targeted delivery and cells separation.

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